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18M2/0206

This is a communication from the examiner in charge of your application.
COMMISSIONER OF PATENTS AND TRADEMARKS

☑ This application has been examined ☐ Responsive to communication filed on _____ ☐ This action is made final.

A shortened statutory period for response to this action is set to expire three (3) month(s), ~~0~~ days from the date of this letter.
Failure to respond within the period for response will cause the application to become abandoned. 35 U.S.C. 133

Part I THE FOLLOWING ATTACHMENT(S) ARE PART OF THIS ACTION:

1. ☑ Notice of References Cited by Examiner, PTO-892.
2. ☑ Notice of Draftsman's Patent Drawing Review, PTO-948.
3. ☑ Notice of Art Cited by Applicant, PTO-1449.
4. ☐ Notice of Informal Patent Application, PTO-152.
5. ☐ Information on How to Effect Drawing Changes, PTO-1474.
6. ☐ _____

Part II SUMMARY OF ACTION

1. ☑ Claims 1-29 are pending in the application.
   Of the above, claims _____ are withdrawn from consideration.
2. ☐ Claims _____ have been cancelled.
3. ☐ Claims _____ are allowed.
4. ☑ Claims 1-29 are rejected.
5. ☐ Claims _____ are objected to.
6. ☐ Claims _____ are subject to restriction or election requirement.
7. ☐ This application has been filed with informal drawings under 37 C.F.R. 1.85 which are acceptable for examination purposes.
8. ☐ Formal drawings are required in response to this Office action.
9. ☐ The corrected or substitute drawings have been received on _____. Under 37 C.F.R. 1.84 these drawings are ☐ acceptable; ☐ not acceptable (see explanation or Notice of Draftsman's Patent Drawing Review, PTO-948).
10. ☐ The proposed additional or substitute sheet(s) of drawings, filed on _____. has (have) been ☐ approved by the examiner; ☐ disapproved by the examiner (see explanation).
11. ☐ The proposed drawing correction, filed _____, has been ☐ approved; ☐ disapproved (see explanation).
12. ☐ Acknowledgement is made of the claim for priority under 35 U.S.C. 119. The certified copy has ☐ been received ☐ not been received ☐ been filed in parent application, serial no. _____; filed on _____.
13. ☐ Since this application apppears to be in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under Ex parte Quayle, 1935 C.D. 11; 453 O.G. 213.
14. ☐ Other

EXAMINER'S ACTION

PTOL-326 (Rev. 2/93)

Claims 1-29 are pending in the instant Application.

The following is a quotation of the first paragraph of 35 U.S.C. § 112:

> The specification shall contain a written description of the invention, and of the manner and process of making and using it, in such full, clear, concise, and exact terms as to enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the same and shall set forth the best mode contemplated by the inventor of carrying out his invention.

The specification is objected to under 35 U.S.C. § 112, first paragraph, as failing to provide an enabling disclosure.

The specification fails to provide an enabling disclosure for how to use the claimed process because it fails to provide adequate guidance and teachings to as to have enabled the artisan to have prepared biologically active fibrinogen molecules. As described by Mosher, (S), fibrinogen was known to have been a member of the set of proteins involved in blood coagulation (see e.g. Table 167-1 at line 1; page 1061) and was known to have been made up a three basic polypeptide chains. Mosher states that "(f)ibrinogen and fibrin monomer are extended trinodular molecules made up of pairs of three polypeptide chains: alpha, beta, and gamma (Fig. 167-2A). The three chains run through half the molecule, that is, through half of the central E nodule and the whole of one of the two peripheral D nodules. The chains are thought to adopt a coiled-spring structure between the E and D modules. This portion of the molecule is particularly susceptible to degradation b the principle fibrinolytic enzyme, plasmin" (see Mosher at page 1063, second column, second full paragraph, lines 1-10). Note also the complex structure of biologically active fibrinogen as represented in Figure 167-2, panel A, wherein it is indicated that proper presentation of biologically active fibrinogen includes the presence of multiple disulfide bonds, and carbohydrate modifications. Thus, biologically active fibrinogen is a large molecule (on the order of 300+ Kd) with a complex structure.

The instantly claimed invention is drawn to two fundamental aspects; (1) methods of preparing fibrinogen in the mammary glands of transgenic animals (claims 1-17, and 20-22); and (2) transgenic animals capable of making fibrinogen (claims 18, 19 and 23-29). The specific teachings present in the as filed specification are limited to guidance in regard to the preparation of transgenic mice and sheep comprising transgenes consisting of fibrinogen encoding genes coupled to a beta-lactoglobulin promoter (see e.g. Example I at page 18, lines 17-24; and Example II). In particular, said transgenes were shown to have been expressed in the mammary glands of transgenic mice. However, reference to the results presented in Example III at page 27, lines 23-33, indicate that while the three transgenes collectively encoding the three subchains of fibrinogen

were expressed in the milk of transgenic mice, no indication is present that said three subchains were correctly processed and assembled into biologically active, mature fibrinogen molecules. The only evidence present in the specification is directed towards an analysis wherein fibrinogen production was monitored by examination of milk under denaturing conditions (see specification at page 27, lines 27-29). Such an analysis fails to demonstrate production of properly processed and mature fibrinogen.

Given the complex structure of fibrinogen (see above), the instants inventions basis in ectopic expression of a fibrinogen subchains, and the fact that the no evidence supports the production of biologically active fibrinogen, coupled with the highly unpredictable nature of transgenic technology, the practitioner would not have accepted that the claimed animals would have been able to have generated biologically active fibrinogen. Therefore, the artisan would not have accepted the teachings present in the as-filed specification as sufficient to have enabled the artisan to have prepared useful fibrinogen compositions.

The specification fails to provide an enabling disclosure for how to use the claimed methods of producing biologically active fibrinogen or for how to use the claimed transgenic animals because the specification fails to provide any guidance in regard to how one would have "recovered" biologically active fibrinogen. Particular limitations present in the claimed methods included the recitation that fibrinogen is "recovered" from milk (see e.g. claim 1). However, the specification offers no teachings in regard to how one would have recovered any fibrinogen that would have been biocompetent.

The following comments are apropos to the scope of the invention as instantly claimed:

The specification fails to provide an enabling disclosure for the preparation of any and all transgenic animals because the teachings present in the specification are limited to guidance in regard to how one would have transgenic mice and sheep. It is noted that Example IV beginning at the bottom of page 27 provides guidance in methods of generating transgenic sheep. However, said guidance in written in the present tense and fails to evidence that one would have been able to have made transgenic sheep that manifested biocompetent fibrinogen in their mammary glands. As noted above, it is questioned whether, even in the case of the production of transgenic mice, the specification would have allowed one to have produced biologically active fibrinogen. This problem is exacerbated when one considers translating the production of fibrinogen to other mammalian systems. As noted by Pursel et al., 1989 (T), a variety of distinct methodologies are

required for the production of transgenic animals with each method having been specifically adapted to particular animals (see e.g. section entitled "Production of Animals" on page 1282, second column). In addition, Hennighausen, 1990 (U) specifically addresses the requirements for using mammary glands for the production of foreign proteins. While general parameters for the design of appropriate systems were known, the design and production of suitable transgenes was not considered predictable (see e.g. first paragraph of section entitled "Targeting Gene Expression to the Mammary Gland" on page 4, second column) and a high degree of unpredictable variability was known to exist when translating the protein production from one animal system to another (see e.g. Hennighausen, (U) at paragraph bridging pages 5 and 6). Given the highly unpredictable nature of the transgenic arts, the lack of guidance in the specification in regard to the selection and design of any and all gene fusions, the nature of the protein sought to be expressed in the instant invention (fibrinogen; see discussion above), and the scope of the claimed invention to include the use of any and all mammals including such diverse organisms as the platypus and whale, the artisan would have been required to have exercised undue experimentation in the practice of the full scope of what is claimed.

For similar reasons to those advanced above, the specification fails to provide an enabling disclosure for the preparation and use of any and all gene constructs comprising any fibrinogen gene. It is acknowledged that fibrinogen genes were known in the art and were considered to have been highly conserved in evolution. Therefore, the instant grounds of objection to the specification and corresponding rejection of the claims advanced below, is not directed to fibrinogen genes, *per se*. However, the claimed methods and animals require the preparation and use of particular gene fusions comprising appropriate promoters and signal (secretion) sequences. As noted by Hennighausen (U), no art recognized means of designing and using general gene fusions in transgenic systems were known (see e.g. first paragraph of section entitled "Targeting Gene Expression to the Mammary Gland" on page 4, second column) and the specification fails to provide general guidance for the production and use of any and all gene fusions in combination with any and all transgenic systems. In the absence of such guidance, the practitioner would have been required to have exercised undue experimentation in the identification, design and utilization of any and all gene fusion constructs potentially useful in combination with transgenic means of producing fibrinogen. Correspondingly, limitation of the scope of the claimed methods and animals to comprise those for which explicit guidance is offered in the specification, (i.e. the use of constructs comprising beta-lactoglobulin promoters for use in transgenic mice), is appropriate.

The specification fails to provide adequate guidance in regard to how to use any and all transgenic non-human mammalian embryos and mammals because the only animals for which guidance is offered in the specification are those that express biologically active fibrinogen in their mammary glands while the scope of the claimed animals includes animals that are not required to be able to so express such fibrinogen. No indication or guidance of how one would have used animals that did not express biologically active fibrinogen in their mammary glands is present in the specification. Therefore, the specification fails to provide adequate guidance in regard to how one would have used the animals within the scope of what is claimed without having had to have exercised undue experimentation. Correspondingly, limitation of the scope of the claimed animals to those that express biologically active fibrinogen in their mammary glands is appropriate.

The specification fails to provide an enabling disclosure for the practice of the full scope of what is claimed because the invention as claimed by claim 15 indicates that a fertilized egg is inserted into the oviduct of a female but no requirement is present that the egg and the female be of the same species. Since the specification fails to provide any guidance in regard to how one would have produced any transgenic animals using heterologous hosts for microinjected eggs, the practitioner would not have been able to have practiced the full scope of what is claimed without having had to have exercised undue experimentation. Inclusion of a same species requirement for a microinjected egg and the host used to allow said egg to develop would obviate the instant objection to the specification and corresponding grounds of rejection of claim 15.

The specification fails to provide an enabling disclosure for how to make and use any and all of the animals within the scope of the invention of claim 18 because said claim merely recites that the heterologous DNA is in the nucleus while the teachings and guidance present in the specification require that the DNA be integrated into the genome and be expressed. How would one use an animal that did not express said DNA? In the absence of such guidance, the practitioner would have been required to have exercised undue experimentation in using all of the animals within the scope of what is claimed. Limitation to those animals that integrate said DNA into their genomes and that express the gene product of said DNA in their mammary glands would obviate the instant objection to the specification and corresponding grounds of rejection of claim 18.

Claims 1-29 are rejected under 35 U.S.C. § 112, first paragraph, for the reasons set forth in the objection to the specification.

Claims 1-15 and 18-25 are rejected under 35 U.S.C. § 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention. It is noted that those claims not specifically indicated below are included in the instant grounds of rejection because they depend from claims which fail to particularly point out and distinctly claim the subject matter which applicant regards as the invention.

Claims 1 and 11 are vague and indefinite because it is unclear as to what is required for the operative linkage between the secretion signal and the fibrinogen chains. Inclusion of language indicating the operation resulting from the linkage would be remedial.

Claims 1 and 20 are similarly unclear because no indication of the operation required for the link to the cited "additional DNA segments" is present in the claim so it is unclear as to what said linkage would require. The use of the phrase "'required' for its expression" is unclear because it is uncertain as to what would be the metes and bounds of the additional DNA segments and whether such segments are required to be "sufficient" for mediating expression in the mammary gland. The claim is also unclear because it is uncertain as to the antecedent of "its" in the phrase "its expression". Does "it" refer to all of the first, second and third segments or is only subsets of said segments?

Claim 5 is vague and indefinite because it is unclear as to the nature of the operative linkage between the segments and the transcription promoter. Recitation of the operation resulting from said linkage would be remedial.

Claim 18 is inaccurate because it indicates that an "embryo" contains within its "nucleus" heterologous DNA. However, an embryo is the term normally applied to any preparturition mammal and may comprise multiple cells with multiple nuclei. An embryo, *per se* does not have any objective antecedent for "a nucleus".

Claim 19 is vague and indefinite because it is unclear as to what would have been considered to have been "recoverable" amounts of human fibrinogen. How much is required for an amount to have been considered "recoverable"?

The following is a quotation of 35 U.S.C. § 103 which forms the basis for all obviousness rejections set forth in this Office action:

> A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art

to which said subject matter pertains. Patentability shall not be negatived by the manner in which the invention was made.

Subject matter developed by another person, which qualifies as prior art only under subsection (f) or (g) of section 102 of this title, shall not preclude patentability under this section where the subject matter and the claimed invention were, at the time the invention was made, owned by the same person or subject to an obligation of assignment to the same person.

This application currently names joint inventors. In considering patentability of the claims under 35 U.S.C. § 103, the examiner presumes that the subject matter of the various claims was commonly owned at the time any inventions covered therein were made absent any evidence to the contrary. Applicant is advised of the obligation under 37 C.F.R. § 1.56 to point out the inventor and invention dates of each claim that was not commonly owned at the time a later invention was made in order for the examiner to consider the applicability of potential 35 U.S.C. § 102(f) or (g) prior art under 35 U.S.C. § 103.

Claims 1-8 and 11-29 are rejected under 35 U.S.C. § 103 as being unpatentable over Meade et al., 1989 (A), Archibald et al., 1990 (A4), and Roy et al., 1991 (A7).

The instant invention is drawn to means of producing recombinant fibrinogens in the mammary glands of transgenic mammals. Particular embodiments of what is claimed include the preparation of nucleic acid constructs comprising human fibrinogen Aα, Bβ, and γ chains, introduction of such into embryos and production of germ line transgenic progeny.

Meade et al. teaches that production of recombinant proteins in the milk of transgenic animals. In particular, Meade et al. states that

> Many important proteins...are large (molecular weights in excess of 30 Kd), secreted, require sulfhydryl bonds to maintain proper folding, are glycosylsated, and are sensitive to proteases. As a result, the recombinant production of such products in prokaryotic cells has proven to be less satisfactory because the desired recombinant proteins are incorrectly processed, lack proper glycosylation or are improperly folded. Accordingly, resort has been had in the production of those recombinant proteins in cultured eukaryotic cells. This technique has proven to be both expensive and often unreliable due the variability of cell culture methods. For example, average yields are 10 mg of recombinant protein per liter of culture media, with resulting cost typically exceeding $1,000 per gram of recombinant protein...
>
> The present invention solves such problems of providing efficient means of producing large quantities of recombinant protein products in the milk of transgenically altered mammals. According to this invention, *a DNA sequence coding for a desired protein is operatively linked in an expression system to a milk specific protein promoter, or any promoter sequence specifically activated in mammary tissue, through a DNA sequence coding for a signal peptide that permits secretion and maturation of the desired protein in the mammary tissue,*

(column 1, lines 32-62, emphasis added.) Meade et al. further teach that such a DNA sequence comprising a gene of interest is transgenically incorporated into a mammalian embryo and the recombinant protein then produced in the milk of the lactating mammal harboring said gene. Meade et al. also disclose that "cows, sheep, goats, mice, oxen, camels and pigs are preferred hosts (see e.g. column 2, lines 53-61). At column 3, lines 1-8, a number of promoter elements useful for mediating expression of a gene of interest in the mammary gland are listed and it is indicated therein that such promoters include the casein promoter and the beta lactoglobulin promoter (see e.g. column 3, line 3). Genes specifically listed by Meade et al. as desirable for production in transgenic mammary glands include coagulation factors VIII and IX, among others (see e.g. column 3, lines 31-40). Note also that Meade et al. specifically disclose art recognized means of preparing transgenic animals and that said means included injection of nucleic acid constructs into fertilized mammalian eggs, passage of the resulted transgene through the germ line and production of desired proteins in milk (see e.g. column 4, lines 32-61). Meade et al. also specifically discloses means and methods of producing recombinant proteins in transgenic mice and transgenic sheep (see e.g. Examples 3 and 4 at columns 6-8). (See also claim 1 of Meade et al. for exemplification of the disclosed process.)

While Meade et al. discloses both general and specific means of producing recombinant proteins in the milk of transgenic mammals, Meade et al. does not teach the preparation of animals transgenic for fibrinogen genes or the use of such animals to produce fibrinogen.

Archibald et al. also teach the utilization of transgenic animals for the production of recombinant proteins of interest as well as teaching that in the design of transgenes, one should include introns so as to maximize gene expression (see e.g. page 3, lines 14-16 and page 5, lines 30-32). Archibald et al. also note the use of a variety of promoters including that of whey acidic protein, alpha-lactalbumin, casein, and beta-lactoglobulin (BLG; see e.g. page 6 at lines 12-14). Note that Archibald et al. specifically note that the use of the BLG promoter in combination with plasma protein encoding genes represents a particularly preferred combination (see e.g. page 7 at lines 14-24).

Roy et al. disclose the production of recombinant human fibrinogen in eukaryotic cells (COS) *in vitro* and that in "two experiments COS-α,β,γ cells...secreted an average of 2.08 μg of fibrinogen in 24 hours..." (see page 4762, first column, fourth paragraph, lines 3-5). Note also that Roy et al. specifically state that "COS cells containing all three fibrinogen chain cDNAs express, assemble, and secrete the chains in a form which is capable of forming a thrombin-induced clot" (sentence bridging first and second columns of page 4762). Thus, Roy et al. disclose

the recombinant production of a biologically (biocompetent) blood protein. See also results presented in Figure 5 and disclosure of human fibrinogen genes in Figure 1.

Roy et al. also disclose that "fibrinogen is a composed of three different polypeptides (Aα, Bβ, and γ) arranged as a dimer with each half-molecule containing a set of each of the chains" (page 4758m, first column, first paragraph of introduction, lines 1-3). Therefore, the stoichiometry of fibrinogen including equal molar amounts of the different polypeptides was known in the art.

As noted above, the use of transgenic animals as bioreactors for the production of recombinant proteins of interest was well known (Meade et al. and Archibald et al.). It was also known that recombinant production of human fibrinogen was desirable. Thus, given the fact that human fibrinogen genes were known as were the parameters for designing of appropriate constructs (promoters such as beta-lactoglobulin, inclusion of a signal sequence, inclusion of introns), it would have been obvious to one of ordinary skill at the time of the invention to have prepared transgenic animals that expressed recombinant human fibrinogen in their mammary glands so as to have produced useful quantities of protein. In regard to the specifically recited limitations wherein it is stated that both male and female transgenic animals would be produced, it is noted that in the production of transgenic animals both sexes are generated. Male animals would have been recognized as useful for breeding purposes and female animals would have been recognized as useful because they have mammary glands for production of recombinant proteins in milk.

In regard to the limitations recited in claims 4 and 14 wherein it is noted that equimolar amounts of the gene fusions of the claimed invention are used, it is noted that given the fact that the stoichiometric amounts of the fibrinogen subchains were known (Roy et al.) to have been equimolar, it would have been *prima facie* obvious to one of ordinary skill in the art at the time of the invention to have used equimolar amounts of DNA in the preparation of the claimed transgenic animals so as to have favored the production of equimolar amounts of protein for assembly into mature fibrinogen.

One would have been motivated to have prepared the claimed transgenic animals for the production of recombinant fibrinogen because the prior art indicated that it was desirable to produce proteins of interest in the mammary glands of transgenic animals and fibrinogen (a blood protein) was considered desirable.

Therefore, for the preceding reasons, the claimed invention would have been *prima facie* obvious to one of ordinary skill in the art at the time the invention was made.

Claims 9 and 10 are rejected under 35 U.S.C. § 103 as being unpatentable over Meade et al., 1989 (A), Archibald et al., 1990 (A4), and Roy et al., 1991 (A7) as applied to claims 1-8 and 11-29 above, and further in view of Chung et al., 1990 (A18) and Lewin, 1983 (R).

The claims under instant consideration are drawn to particular embodiments of the methods of preparing fibrinogen wherein the Bβ-fibrinogen chain contained in the second DNA segment comprises nucleic acids depicted by Seq. ID. number 3 and that begins at either nucleotide 470 (claim 9) or nucleotide 512 (claim 10) and ends at nucleotide 8100. Reference to the specification at page 8, lines 4 and 5 and comparison of the nucleotide sequence presented in Seq. ID. number 3, shows that said sequence is identical to that disclosed by Chung et al. in Figure 4 wherein the nucleotide sequence of the gene coding for the Bβ chain of human fibrinogen is disclosed.

Chung et al. teaches the sequence of human Bβ-fibrinogen within the body of figure 4. Reference to said Figure of Chung et al. at page 43 reveals that the accepted amino terminal amino acid of said human fibrinogen is encoded by nucleotide number 560 and is a Gln residue. However, translational initiation occurs at methionine (Met) residues. Therefore, one would have known that if one desired production of a protein, it was necessary to have supplied a 5' nucleotide encoding a Met residue. Since prepro- forms of proteins were known in the art, one would have inspected the known nucleotide sequence of Bβ-fibrinogen to find a Met residue encoded and in-frame with the accepted Gln start of Bβ-fibrinogen. Inspection of the sequence presented by Chung et al. in Figure 4 reveals two such Met residues. One is located at position 470-472 and another at position 512-514.

Lewin teaches the well known fact that initiation of protein synthesis in both prokaryotic and eukaryotic systems requires the presence of a methionine (Met) codon (see e.g. page 94 , first column, first and second full paragraphs).

Since the requirements for the initiation of protein synthesis (i.e. the presence of an Met encoding codon) were well recognized, absent unexpected results obtained from the utilization of any particular nucleic acid sequence, it would have been obvious to one of ordinary skill at the time of the invention to have designed expression constructs so as to have provided an initiator methionine residue.

One would have been motivated to select such initiation sites for use in expression constructs because one would have known that such sites were required for protein synthesis.

Therefore, for the preceding reasons, the claimed invention would have been *prima facie* obvious to one of ordinary skill in the art at the time the invention was made.

No claims are allowable.

Any inquiry concerning this communication or earlier communications from the examiner should be directed to Brian R. Stanton whose telephone number is (703) 308-2801. The examiner can normally be reached Monday-Thursday from 6:30 AM to 5:00 PM.

Any inquiry of a general nature or relating to the status of this application should be directed to the Group receptionist whose telephone number is (703) 308-0196.

Brian R. Stanton

Brian R. Stanton, Ph.D.
Patent Examiner
Art Unit 1804

28 January 1995